

Localization of a Highly Conserved Human Potassium Channel Gene (NGK2-KV4; KCNC1) to Chromosome 11p15

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Several genes (the Shaker or Sh gene family) encoding components of voltage-gated K⁺ channels have been identified in various species. Based on sequence similarities Sh genes are classified into four groups or subfamilies. Mammalian genes of each one of these subfamilies also show high levels of sequence similarity to one of four related *Drosophila* genes: Shaker, Shab, Shaw, and Shal. Here we report the isolation of human cDNAs for a Shaw-related product (NGK2, KV3.1a) previously identified in rat and mice. A comparison of the nucleotide and deduced amino acid sequence of NGK2 in rodents and humans shows that this product is highly conserved in mammals; the human NGK2 protein shows over 99% amino acid sequence identity to its rodent homologue. The gene (NGK2-KV4; KCNC1) encoding NGK2 was mapped to human chromosome 11p15 by fluorescence *in situ* hybridization with the human NGK2 cDNAs. © 1993 Academic Press, Inc.

INTRODUCTION

Potassium channels are ubiquitous membrane proteins that play important roles in many types of cellular functions (Hille, 1992; Petersen and Maruyama, 1984; Lewis and Cahalan, 1988; Rudy, 1988; Dreyer, 1990; Ashcroft, 1988; Petersen and Findlay, 1987; Rudy *et al.*, 1991a). For example, potassium channels are important in neuronal function, they help set the resting potential and the degree of excitability of the membrane, they influence action potential waveforms and firing patterns (Hille, 1992; Adams and Galvan, 1986; Thompson and Aldrich, 1980; Llinas, 1984, 1988), and they modulate synaptic activity (Siegelbaum *et al.*, 1982; Klein *et al.*, 1982). K⁺ channels are present in T-lymphocytes where they may play a role in stimulus-dependent activation (Cahalan *et al.*, 1987; Lewis and Cahalan, 1988) and in osmotic regulation (Grinstein and Smith, 1990), and also in pancreatic β cells where they play a role in the regulation of insulin secretion (Henquin and Meissner, 1984; Ashcroft and Rorsman, 1989; Ashcroft, 1988; Fa-

therazi and Cook, 1991; Petersen and Findlay, 1987). Thus, potassium channel genes are potential candidates for a wide variety of genetic diseases ranging from neuromuscular disorders to tumorigenesis. For example, several mutants in *Drosophila* with neurological abnormalities have defects in K⁺ channel genes (Ganetzky and Wu, 1986; Jan and Jan, 1990; Lichtenhagen *et al.*, 1990; Warmke *et al.*, 1991; Atkinson *et al.*, 1991); mice with mutations of the *lpr* gene have a generalized lymphoproliferation and show an altered distribution of voltage-gated potassium channels in the abnormal T lymphocytes (Chandy *et al.*, 1986). The determination of the chromosomal localization of potassium channel genes is an important first step in discovering their potential involvement in human genetic disease.

Potassium channels are also extremely diverse, allowing for a fine control of their effects in distinct cells or in different circumstances (Hille, 1992; Latorre *et al.*, 1989; Rudy, 1988). One gene family, the Shaker or Sh gene family, encoding about 20 different mRNAs in mammals, has been recently characterized (reviewed in Pongs, 1989; Jan and Jan, 1990; Rudy *et al.*, 1991a; Perney and Kaczmarek, 1991; MacKinnon, 1991). Although functional expression studies indicate that it is very likely that Sh mRNAs encode components of voltage-gated K⁺ channels, one of the functional classes of K⁺ channels, in most cases we do not know yet which are the native channels containing these components (Rudy *et al.*, 1991a). Moreover, the role in cell and tissue function of many of these proteins both in excitable and non-excitable tissues also remains to be elucidated. The discovery of genetic mutants of these genes in humans and mice may help in this process. The organization of this gene family in the genome is also of interest and may shed light on their relationships to each other and perhaps to other genes.

Based on sequence similarities, and hence probable evolutionary relationships, the Sh family is divided into four subfamilies (ShI, ShII, ShIII, and ShIV). A member of a subfamily in mammals is more similar to one of four Shaker-like *Drosophila* genes (Shaker, Shab, Shaw, and Shal respectively) than to a mammalian member of a different subfamily (Wei *et al.*, 1990; Jan and Jan, 1990;

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Rudy *et al.*, 1991a; Perney and Kaczmarek, 1991). We have cloned a cDNA encoding a human ShIII or Shaw-related potassium channel RNA (human NGK2²) previously cloned from mouse (NGK2, Yokoyama *et al.*, 1989) and rat (rat KShIIIB or rat NGK2, Vega-Saenz de Miera *et al.*, 1990; Sen *et al.*, 1991; Luneau *et al.*, 1991a; Rudy *et al.*, 1991b). In rat, a second product (KV4, KV3.1b, or KCNC1b) of the gene (NGK2-KV4, gene symbol: KCNC1) encoding NGK2 is generated by alternative splicing at the 3' end, resulting in proteins with different carboxyl ends (Luneau *et al.*, 1991a). In the KV4 protein the last 10 amino acids of NGK2 are replaced by 84 residues (Luneau *et al.*, 1991a).

Here we report a comparison of the nucleotide and deduced amino acid sequences of NGK2 in rat and human. We find that the sequence of this potassium channel gene product is extremely well conserved, at both the nucleotide and the amino acid levels. We also report the chromosomal location of this gene on human chromosomes.

MATERIALS AND METHODS

Screening of cDNA libraries. Two cDNA libraries in λ gt11 derived from human fetal spinal cord and human brain stem (gifts from Dr. Celia Campagnoni, UCLA, and Dr. Carmie Puckett, California Institute of Technology) were screened with probes derived from rat ShIII cDNAs as previously described (Rudy *et al.*, 1991b). DNA was extracted from purified positive phage plaques after amplification. The DNA was cut with *EcoRI* and the inserts were gel purified and subcloned into pBluescript (Stratagene). Three overlapping cDNA clones (4-2, ~1.4 kb; 23-3, ~600 kb; and 38-3, ~1.5 kb) were isolated and characterized. Clones 4-2 and 23-3 were utilized to derive the sequences shown in Fig. 1. Clone 4-2 contains the first 1437 bases (–68 to 1369 in Fig. 1a), and clone 23-3 the last 600 nucleotides. The initial 700 bases of clone 38-3 show no similarity to the other two clones, the last 799 bases are identical to the sequence of 4-2 from position 570 (in Fig. 1a) to the 3' end (position 1369). Position 570 corresponds to an intron-exon boundary in the rat NGK2-KV4 gene (Luneau *et al.*, 1991a; Vega-Saenz de Miera and Rudy, unpublished observations) and the sequence around this position in 38-3 (ATCCCCACAGTA) agrees well with the consensus sequence for a splice acceptor site (YYNCAG; Padgett *et al.*, 1986). Moreover, there is a significant level of sequence similarity between the sequence of the first 700 bases of 38-3 and the sequence of the corresponding intron in rat (Vega-Saenz de Miera and Rudy, unpublished observations). Therefore, we conclude that the first 700 bases of the 38-3 cDNA correspond to intervening sequence and that the clone was derived from the priming of an incompletely processed pre-mRNA during library construction. Sequences were obtained by the dideoxynucleotide chain-termination method (Sanger *et al.*, 1977) as previously described (Rudy *et al.*, 1991b).

In situ hybridization. The chromosomal localization of NGK2 was determined by fluorescence *in situ* hybridization as previously described (Ried *et al.*, 1990; Rudy *et al.*, 1991b). Metaphase spreads were obtained from peripheral blood lymphocytes of a healthy donor and cultured following the methotrexate synchronization procedure described by Yunis (1976). *In situ* hybridization was performed following standard protocols (e.g., Lichter *et al.*, 1990; Ried *et al.*, 1992a). Briefly, 40 ng of each of the three overlapping cDNA clones (4-2, 23-3, and 38-3), labeled with biotin-11-dUTP, was combined and precipi-

tated with 40 ng of digoxigenin-labeled *Alu*-PCR products (Baldini and Ward, 1991) in the presence of 5 μ g of salmon sperm DNA and yeast RNA, respectively. The DNA was resuspended in 10 μ l of hybridization solution (50% formamide, 2 \times SSC, 10% dextran sulfate) and denatured at 76°C for 5 min. The probe DNA was then applied to the chromosome preparations which were denatured separately (2 min, 80°C, followed by a dehydration through an ethanol series). A coverslip (18 \times 18 mm²) was added and sealed with rubber cement. Hybridization took place overnight at 37°C. After three 5-min washes in 50% formamide, 2 \times SSC at 42°C, followed by three washes in 0.2 \times SSC at 60°C, a blocking step was included with 3% bovine serum albumin (BSA) in 4 \times SSC. The probe sequences were detected with avidin-DCS-fluorescein isothiocyanate (Vector laboratories). The *Alu*-PCR probes were visualized using anti-digoxigenin conjugated to rhodamine (Boehringer-Mannheim). Both detection steps were performed simultaneously in 1% BSA in 4 \times SSC for 30 min at 37°C. After three washes in 4 \times SSC, 1% Tween at 37°C, the specimens were imbedded in antifade (DABCO) containing DAPI (150 ng/ μ l) as a counterstain.

The fluorescence signals were visualized using a cooled CCD camera (PM512, Photometrics, Tucson, AZ). Gray-scale images were obtained sequentially for fluorescein, rhodamine, and DAPI with precision filter sets, manufactured by C. Zeiss, Germany, to minimize image shifts. With the aid of custom computer software, the gray-scale images were pseudocolored and merged (for details see Ried *et al.*, 1992b). Photographs were taken using Kodak 100 HC color slide films.

RESULTS AND DISCUSSION

NGK2 Is Extremely Well Conserved between Rodents and Humans

A comparison of the nucleotide and deduced amino acid sequences of the rat and the human NGK2 gene products is shown in Figs. 1a and 1b, respectively. The NGK2 protein is extremely well conserved with over 99% amino acid identity: only one amino acid conservative substitution is seen in the two species. The nucleotide sequence of the coding region is also extremely well conserved with over 90% identity. This similarity is higher than that required to maintain conservation of the protein, but the basis or the significance of this is not clear at this stage. The degree of conservation of the protein sequence is similar to that seen in the histones, the most conserved proteins known. However, the nucleotide conservation is even higher than that seen in histone genes.

The NGK2-KV4 gene is one of four closely related genes (the ShIII subfamily; Rudy *et al.*, 1991a; Vega-Saenz de Miera *et al.*, 1992). Each ShIII gene generates more than one product by alternative splicing; counting those identified in rat and human, there is a total of 10 different ShIII transcripts known (Yokoyama *et al.*, 1989; McCormack *et al.*, 1990a; Luneau *et al.*, 1991a; Rudy *et al.*, 1991b; Schroter *et al.*, 1991; Luneau *et al.*, 1991b; Ghanshani *et al.*, 1992; Rudy *et al.*, 1992; Vega-Saenz de Miera *et al.*, 1992). ShIII proteins show about 70% amino acid sequence identity (Rudy *et al.*, 1991a; Vega-Saenz de Miera *et al.*, 1992). Most of the differences are localized to parts of the amino end region preceding the first membrane spanning domain, the carboxyl domain following the membrane portion of the polypeptide, and the linkers between the first and the

² NGK2 corresponds to KShIIIB in some of our previous publications and to KV3.1a in the nomenclature of Chandry *et al.* (1991). The Human Genome Nomenclature Committee designation for this product is KCNC1a (see also Table 1).

a			
Human NGK2			
Rat NGK2			
CCGCGGTCTCTGTGCCCCGACGGCTGGGGGAGGGGGAAGAGGCCCTGCGCCCCCTCCCGTC-C		GG	
GGCGCGCCAGCGACACCATGGAGCGCGCCAGGGGGAGTTGGCGCGGGGAGGGGGCGCATGG	4	GAGTTCTCATGCGTGTATCTTCTGCCCAACAGGTAGAGTTATCAAGAACTCGCTCAACATATTG	844
CAACTCC--T-GCGGAG-TCCCAT-G-T-TCG-T--CCGCGCAT--TAA-G--C-C-----	4	-----TG-----T-----G-----C-----T-----	844
GCCAAAGGAGACGAGAGCGAGCGCATCGTATCAACGTGGCGGCACGCGCCACAGACGTACCGCTCGAC	74	ACTTTGTGCCATCTGCCCCCTTACCTGGAGGTGGGCTGAGCGGGCTGTCTTCCAAAGGACGCCAAGGA	914
-----C-----T-----G-----G-----A-----T-----C-----A-----T-----	74	-----C-----T-----C-----A-----T-----	914
CCTGCGACAGCTGCCCCGACGCGGCTCGCTGGCTGGCGGAGCCGACGCCACAGCCACTTCGACTAT	144	CGTGTGGGCTTCTGCGGCTGCTCGCTTCGTGCGCATCTTGCGCATCTTTAAGCTGACCGCCACTT	984
-----T-----G-----G-----C-----C-----T-----C-----T-----T-----	144	-----A-----T-----C-----C-----T-----C-----T-----T-----	984
GACCCGCTGCTGACGAGTTCTTCTTGACCGCACCCGCGCTTTCGCGACATCTGAACACTAC	214	GTGGGCTGCGGGCTCTGGGCGACACGCTCCGAGCCAGCACCAGATTCGTGCTCATCATCTTCC	1,054
-----C-----G-----T-----G-----C-----T-----T-----T-----	214	-----T-----T-----T-----T-----T-----T-----	1,054
GCACGGGAGCTGACTGCCAGCCGACGTGTGCGGGCGCTTACGAGGAGGAGCTGGCTTCTGGGG	284	TGGCTTGGGCTGCTGATCTTCCCACTGATCTACTACGCCGAGAGGATAGGGGACAGCCCAATGA	1,124
-----C-----A-----G-----G-----T-----T-----T-----T-----T-----T-----	284	-----C-----T-----C-----T-----T-----T-----T-----T-----T-----	1,124
CATCGACGAGACCGTGGAGCCCTGCTGTGATGACGTACCGCGACGCCGACGCCGAGGAGGCT	354	CCCCAGTGGCAGTGAGCACACGACITTAAGAACATCCCATCGGCTTCTGGTGGGCGGTGGTCACATG	1,194
-----G-----T-----T-----T-----T-----T-----T-----T-----T-----	354	-----C-----C-----A-----A-----A-----A-----T-----T-----T-----T-----	1,194
CTGGACAGCTTCGGCGGCGCTCTCTGGACACAGCGCCGACGCGGACGCCGACGCCCTGGCGACT	424	ACGACCTGGGCTATGAGACATGTACCCGAGAGCTGGTCCGGATGCTGGTGGGCGCTCTGTGTGCG	1,264
-----T-----T-----G-----C-----T-----T-----T-----T-----T-----T-----	424	-----A-----T-----T-----T-----T-----T-----T-----T-----T-----	1,264
CGGGCGAGCGGAGGACGAGCTGGAGATGACCAAGCGCTGGCGCTCAGTGACTCCCGGATGGCGGCG	494	TGGCGGCTGCTCACCATCGCATGCCCGTCCCGTCTCGTGAACAATTTCGGATGTATTACTCTT	1,334
-----A-----A-----T-----A-----T-----A-----T-----A-----T-----	494	-----T-----T-----G-----T-----G-----T-----G-----T-----T-----	1,334
TGGCGGCTTTTGGCGGCGTGGGAGCGCGCATCTGGCGCTCTTGAAGACCGTACTCTGCCGCTAC	564	AGCCATGGCTAAGCAGAACTACCAAGAAAAAAGAGCATATTCGCGGCCACCGCAGCTGGGATCT	1,404
-----C-----A-----A-----A-----A-----A-----A-----A-----A-----	564	-----C-----A-----A-----A-----A-----A-----A-----A-----A-----	1,404
GCGCGATATGCGCTTCCCTCTCTTCTATCTGCTCCATCACCACCTTCTGCTGGAGACC	634	CCCAATTATTTAATCTGTCTGAACCTCCACACCACTACTAGAGTGACATGTCCGCTGGGCC	1,474
-----C-----T-----C-----T-----T-----T-----T-----T-----T-----	634	-----A-----T-----T-----T-----T-----T-----T-----T-----T-----	1,474
ACGAGCGCTTCAACCCATCGTGAACAAGAGGAGATCGAGAACGTTTCGAATGGACGAGTGGCTA	704	AGGAAGAAATTTAGAAATTAACAGAGCAGTGAAGAACTCTCAGAGCATGTGATCTGA	1,536
-----A-----A-----T-----A-----A-----A-----A-----A-----	704	-----A-----A-----A-----A-----A-----A-----A-----A-----	1,536
CTACCGGAGGCGGAGACGAGGCGCTTCTTACCTACATCGAGGCGCTGTGTGCTGTTACCTTC	774		
-----A-----G-----C-----T-----T-----C-----T-----C-----T-----	774		
b			
Human NGK2	MGQGDSEIRIVNVGTRHQTYSRLRLPGLRLAWLAEPDAHSFVDYDPRADFFDRHPGVFAHILNY	70	
Rat NGK2	-----	70	
Human NGK2	YRTGLKHPADVCPLEYEELAFWIDETDVEPCWMTYRQHRDAEALDSFGGAPLNSADDADADGGG	140	
Rat NGK2	-----	140	
Human NGK2	DSGDGEDELEMTKRLALSDSPDGRPGFWRRWQPRIWALFEDPYSSRYARYVAFSLFFILVSTITFCLE	210	S1
Rat NGK2	-----	210	
Human NGK2	THERFNPIVNKTEIENVNRGTQVRYREAEAFETYIEGVVWFTEFLMRVIFCPNKVEFIKNSLNI	280	S2
Rat NGK2	-----V-----	280	
Human NGK2	IDFVAILPFYLEVGLSGLSSKAADVLGFLRVRFVIRILRIFKLTRHFVGLRVLGHTLRASNEFLLII	350	S3
Rat NGK2	-----	350	
Human NGK2	FLALGVLIFATMIYYAERIGAQPNDPSASEHFKNPIGFWAVVTMTTLGYGDMYPQTWSGMLVGALC	420	S4
Rat NGK2	-----	420	
Human NGK2	ALAGVI.TIAMPVPVTVNNFMYSLAMAKQKPKKKKHIPRPQLGSPNYCKSVVNSPHSTQSDTCPL	490	S5
Rat NGK2	-----	490	
Human NGK2	AQEEILEINRAGRKLPGMSI*	511	H5
Rat NGK2	-----	511	

FIG. 1. (a) Comparison of the nucleotide sequences of human and rat NGK2 cDNAs. Rat NGK2 (rat KShIIIB) was cloned from PC12 cell libraries (Vega-Saenz de Miera *et al.*, 1991; Rudy *et al.*, 1991b). Nucleotides in rat identical to those in human are shown with a dash. The numbers indicate the nucleotide positions with the chosen initiation codon as 1. The first base of each codon is indicated with a dot (starting at the putative start codon). The coding portion of the cDNAs is highly conserved and most substitutions are in third positions. The stop codon is shown by an asterisk. An arrow indicates the point of divergence between NGK2 and KV4 (Luneau *et al.*, 1991a). (b) Comparison of the predicted amino acid sequences of the human and rat NGK2 proteins. Amino acids in rat identical to those in human are shown with a dash. The rat (Rudy *et al.*, 1991b; Luneau *et al.*, 1991a) and the mouse (Yokoyama *et al.*, 1989) NGK2 proteins have identical sequences. The hydrophobic domains (S1-S3, S5, H5, and S6) and the S4 motif (Jan and Jan, 1990; Rudy *et al.*, 1991a; Perney and Kaczmarek, 1991) are overlined. An arrow indicates the point of divergence between NGK2 and KV4 (Luneau *et al.*, 1991a). The stop codon is shown by an asterisk.

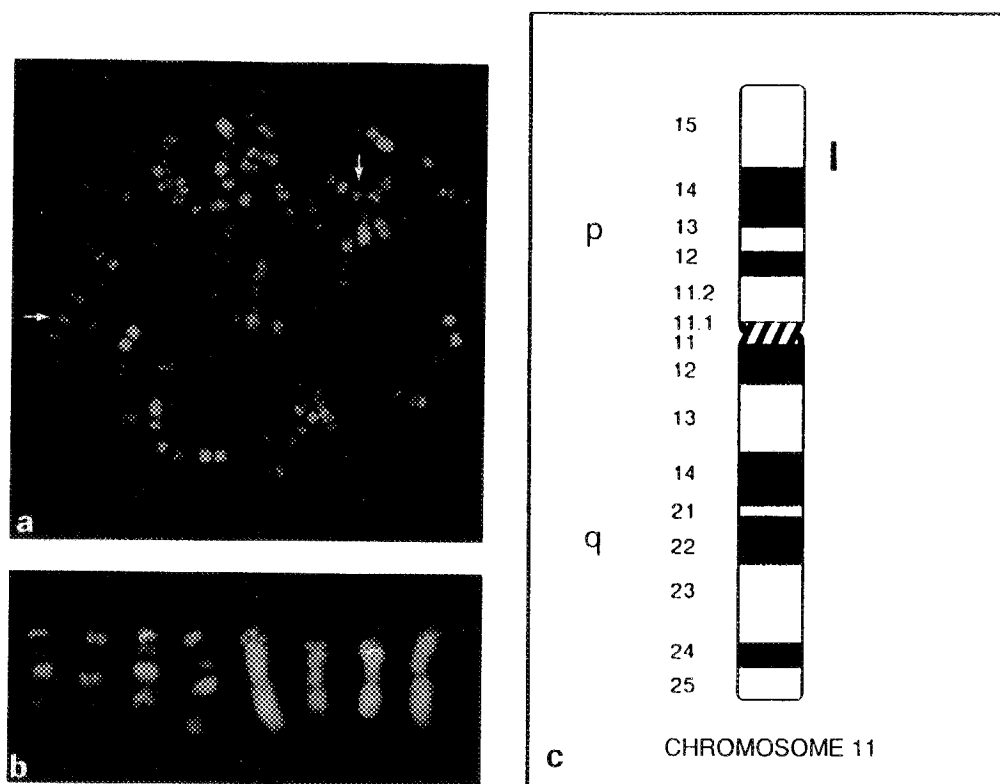


FIG. 2. Chromosomal mapping of NGK2 cDNAs. (a) Human metaphase spread after hybridization with the cDNA clones specific for a potassium channel (NGK2). The R-banding pattern, produced by cohybridizing *Alu*-PCR products with the cDNA probe, is pseudocolored green. The chromosomal signal on 11p15 (arrows) is pseudocolored red, for best photographic contrast. (b) Comparison of a R-like hybridization banding (green) and the DAPI banding (G-like, blue). The chromosomal map position of the gene for the potassium channel on 11p15 is consistent. (c) Idiogram of a G-banded chromosome 11 with the map position of the cDNA clone NGK2 denoted by a bar.

second and between the second and the third membrane spanning domains. The conservation seen here throughout the length of the protein suggests that the portions of the polypeptide that are not conserved among different members of the subfamily are important for specific roles of the NGK2 protein. Particularly interesting is the comparison with the products of the rat KShIIIA gene, a ShIII gene that generates three or four products by alternative splicing (Luneau *et al.*, 1991b; Rudy *et al.*, 1992). KShIIIA, NGK2, and KV4 transcripts express in *Xenopus* oocytes currents that are very similar in voltage dependence, kinetics, and pharmacology (Yokoyama *et al.*, 1989; McCormack *et al.*, 1990a; Luneau *et al.*, 1991a,b; Rudy *et al.*, 1992). Portions of the sequence of a KShIIIA protein are also known in human (Vega-Saenz de Miera *et al.*, 1992; unpublished observations) and are identical to its counterpart in rat, suggesting very strong selection for the differences in structure and function of the protein products of the two genes.

The functional differences between KShIIIA and NGK2-KV4 transcripts remain to be found; however, *in situ* hybridization studies in rat brain demonstrate very different patterns of expression; KShIIIA mRNAs are particularly abundant in the thalamus, but NGK2-KV4 mRNAs are present mainly in the cerebellar cortex (Rudy *et al.*, 1992). NGK2 is also expressed in PC12 pheochromocytoma cells (Vega-Saenz de Miera *et al.*,

1991) and in NG108-15 neuroblastoma-glioma hybrid cells (Yokoyama *et al.*, 1989).

Localization of the NGK2 Gene in Human Chromosomes

The chromosomal mapping position of the cDNA clones specific for the gene NGK2 was determined by fluorescence *in situ* hybridization. Recent improvements of this technique, in particular with respect to detection sensitivity, allow for the visualization of target sequences smaller than 1 kb (e.g., Najfeld *et al.*, 1992). This is mainly due to improved *in situ* hybridization protocols and to digital imaging devices, such as the cooled CCD camera used in our experiments. Arguably, having the technical tools for hybridizing cDNA clones is of particular interest for gene mapping studies and the generation of sequence tagged sites, since the chromosomal map position refers to an expressed gene. Additionally, the search for genetic disease loci is far more streamlined, since the chromosomal map position of candidate cDNA clones for a particular disease can be compared with linkage data without the necessity of isolating genomic DNA clones for *in situ* hybridization experiments.

Recently developed banding techniques, which allow for the simultaneous visualization of chromosome banding patterns and *in situ* hybridization signals with different fluorochromes, result in a direct assignment of probe locations with respect to cytogenetically well-de-

TABLE 1
Human Chromosomal Location of Sh K⁺ Channel Genes

Human Genome Nomenclature Committee name	Name used in mapping paper	Name according to the nomenclature of Chandy <i>et al.</i> (1991)	Other names ^a	Chromosomal location	Mapping references ^b
Subfamily I					
KCNA1	KV1.1	KV1.1	RCK1, RBK1, MBK1, MK1, HuK1	12	McPherson <i>et al.</i> (1991)
KCNA2	MK2	KV1.2	RBK2, RCK5, NGK1, HuKIV	12	Grissmer <i>et al.</i> (1990)
KCNA3	MK3, HLK3, RCK3	KV1.3	KV3, RGK5, HuKIII, HPCN3	13 (MK3); 1p13.3 (HLK3); 1p21 (RCK3)	Grissmer <i>et al.</i> (1990) (MK3); Attali <i>et al.</i> (1992) (HLK3); Ried and Rudy (RCK3, unpublished)
KCNA4	HPCN2, KV1.4 HK1	KV1.4	RCK4, RHK1, HuKII,	11q13-q14 (HPCN2) 11p14.1 (HK1)	Philipson <i>et al.</i> (1992) (HPCN2) Gessler <i>et al.</i> (1992) (HK1)
KCNA5	KCNA1	KV1.5	KV1, HPCN1, HK2	12p	Curran <i>et al.</i> (1992)
KCNA6	—	KV1.6	KV2, RCK2, HBK2	—	—
KCNA7	MK4; K(0)1.7	K(0)1.7	MK6, RK6, HaK6	19	McPherson <i>et al.</i> (1991)
Subfamily II					
KCNB1	—	KV2.1	DRK1, mShab	—	—
KCNB2	—	KV2.2	cDRK1	—	—
Subfamily III^c					
KCNC1	NGK2-KV4	KV3.1	NGK2, KV4, KShIIIB	11p15	This paper
KCNC2	KShIIIA	KV3.2	RKShIIIA	19	Unpublished observation
KCNC3	KV3.3	KV3.3	KShIIID	19	Ghanshani <i>et al.</i> (1992)
KCNC4	KShIIIC	KV3.4	Raw3	1p21	Rudy <i>et al.</i> (1991b)
Subfamily IV					
KCND1	—	KV4.1	mShal 1, KShIVA	—	—
KCND2	—	KV4.2	RK5, Rat Shal 1	—	—
KCND3	—	KV4.3	KShIVB	—	—

^a Names *other* than those shown in previous columns given to the gene or its products in papers where their identification in rat, mouse, or human was reported. These names are often used in the channel literature. References to these articles are available from the corresponding author upon request.

^b When there is more than one paper reporting the same chromosomal location, only the first publication, or that giving the most detailed location, is cited.

^c Multiple transcripts are generated from genes in this subfamily by alternative splicing.

finer bands (Baldini and Ward, 1991; for review see Arnold *et al.*, 1992). Here we used an R-banding from *Alu*-PCR products and a G-banding, which was produced by DAPI, to determine the chromosomal map position of three overlapping cDNA clones specific for the highly conserved potassium channel NGK2. Human NGK2 maps to human chromosome 11p15 (Fig. 2). Eight metaphases were investigated: five of them revealed hybridization signals on both chromatids of the two chromosome 11 homologues. Two metaphases showed signals only on one chromatid or only on one homologue. However, one metaphase did not display any hybridization signal. The band assignment was facilitated considerably by detecting the hybridization banding pattern with a second fluorochrome. The fact that signals were not observed on different locations argues strongly for the specificity of the hybridization. Furthermore, all other known ShIII genes map to different chromosomes (see Table 1). Cross-hybridization between ShIII probes and Sh genes of the other subfamilies is very unlikely (aa sequence identity $\leq 40\%$) under the hybridization conditions used here.

Several Sh genes, including the other three known ShIII genes, have been mapped with various degrees of resolution (Table 1). Although it is believed that the four ShIII genes arose by gene duplication from a common

ancestor, they are distributed on at least three separate chromosomes in man. On the other hand, several ShI genes have been mapped to chromosome 12. It is not yet known, however, if they are all present in a single cluster.

The NGK2-KV4 gene maps to a chromosomal region (11p15) that has been extensively studied (Junien *et al.*, 1992) and shown to harbor several important disease genes, including those associated with Beckwith-Wiedemann syndrome, T-cell leukemias, Type-I diabetes mellitus, and Wilms tumor (Junien *et al.*, 1992; Julier *et al.*, 1991). However, more detailed mapping and tissue expression studies will be required to further assess the possible involvement of the NGK2-KV4 gene in diseases associated with 11p15. Particularly interesting is the possible association of the NGK2-KV4 gene with long QT syndrome, an inherited cardiovascular disease in human recently localized to chromosome 11p15 (Keating *et al.*, 1991). In addition, the NGK2-KV4 gene maps to a chromosomal region showing synteny with a region in chromosome 7 in mice where several behavioral mutants have been localized (Lyon and Searle, 1989).

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